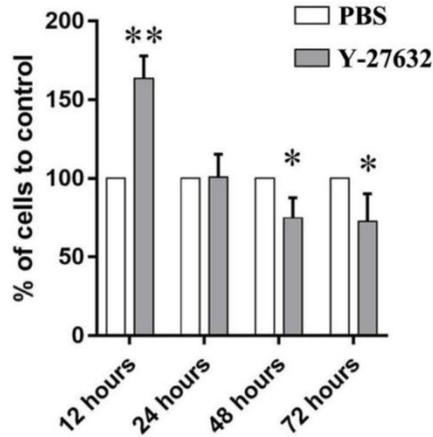
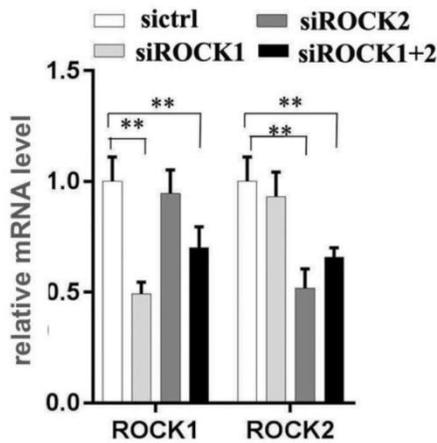


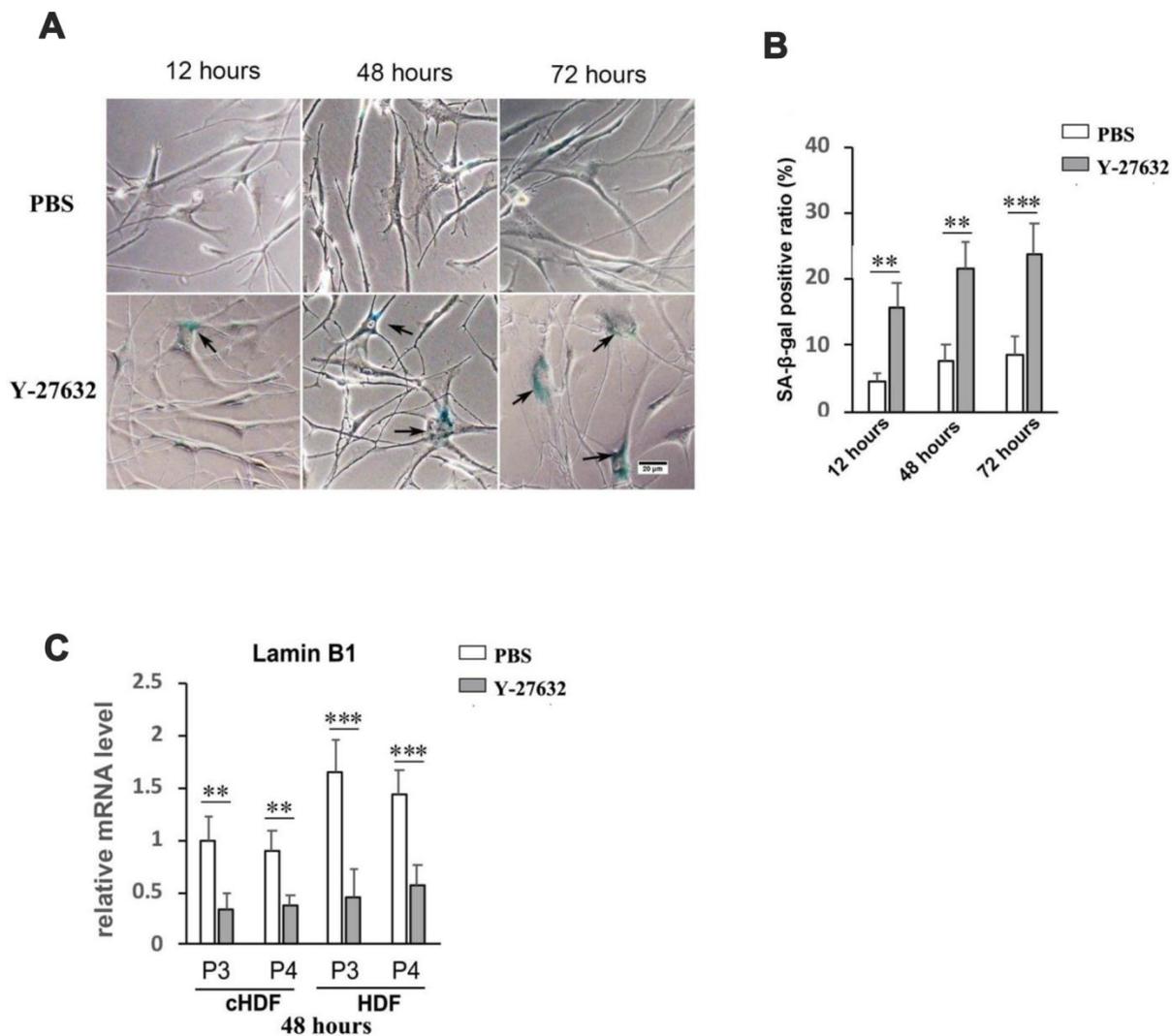
## SUPPLEMENTARY FIGURES



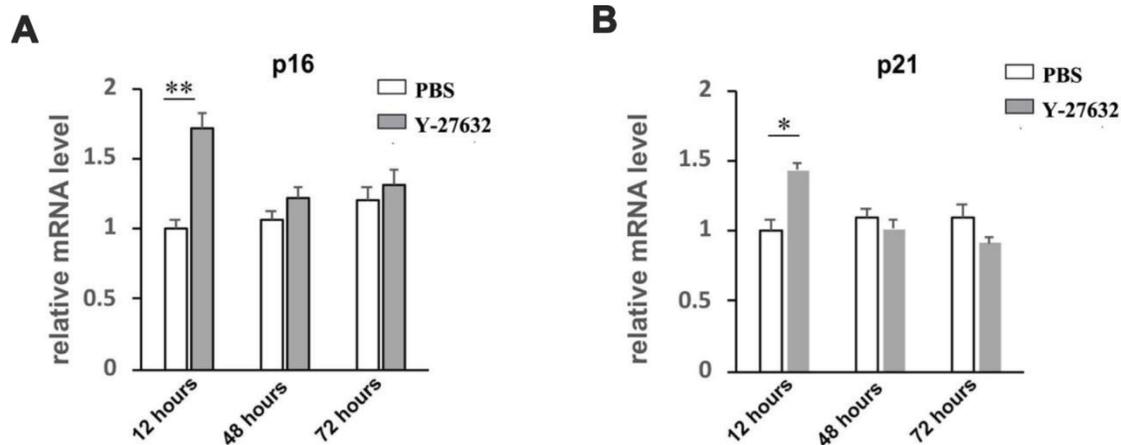
**Supplementary Figure 1. Prolonged treatment with Y-27632 inhibits the growth of commercial HDFs.** Commercial HDFs were cultured under the same conditions as described for Figure 1A, and cells were collected at the indicated times for analysis of cell proliferation using a CCK8 kit; the percentage of cell growth was calculated relative to the corresponding control (PBS) as 100.



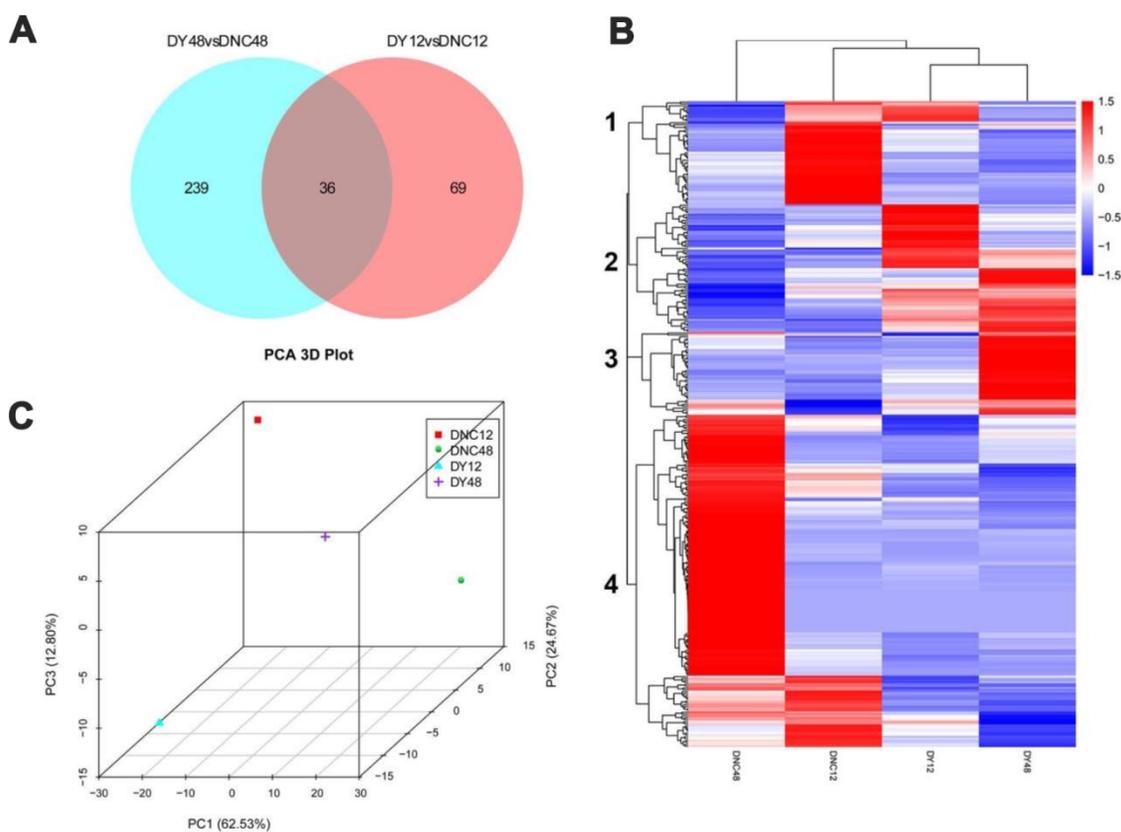
**Supplementary Figure 2. ROCK1/2 expression was efficiently knocked down in HDFs by transfection of the corresponding siRNAs.** HDFs were transfected with siRNAs specific for ROCK1 (siROCK1), ROCK2 (siROCK2) or both ROCK1 and ROCK2 (siROCK1+2); control HDFs were transfected with a scrambled siRNA (sictrl), and cells were collected at 48 h after transfection to detect the expression of ROCK1 and ROCK2 by RT-qPCR analysis.



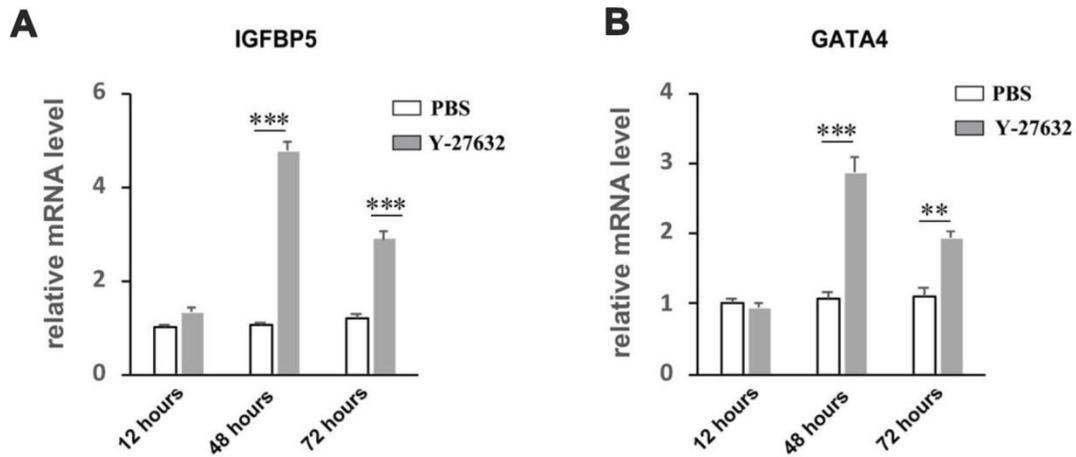
**Supplementary Figure 3. Prolonged treatment with Y-27632 enhances the senescence of commercial HDFs.** (A, B) Commercial HDFs (cHDFs) were treated with PBS or 10  $\mu$ M Y-27632, and then were fixed at the indicated times and analyzed using a SA- $\beta$ -gal staining kit to detect senescent cells. scale bar = 20  $\mu$ m. (B) Quantification of (A), the percentage of SA- $\beta$ -gal-positive cells (arrows, blue) was calculated based on counting a total of 500 cells. (C) Passage 3 or passage 4 commercial HDFs (cHDFs) and our own cloned HDFs (HDFs) were treated with PBS or 10  $\mu$ M Y-27632 for 48 h, after which they were collected for analysis of lamin B1 expression by RT-qPCR analysis.



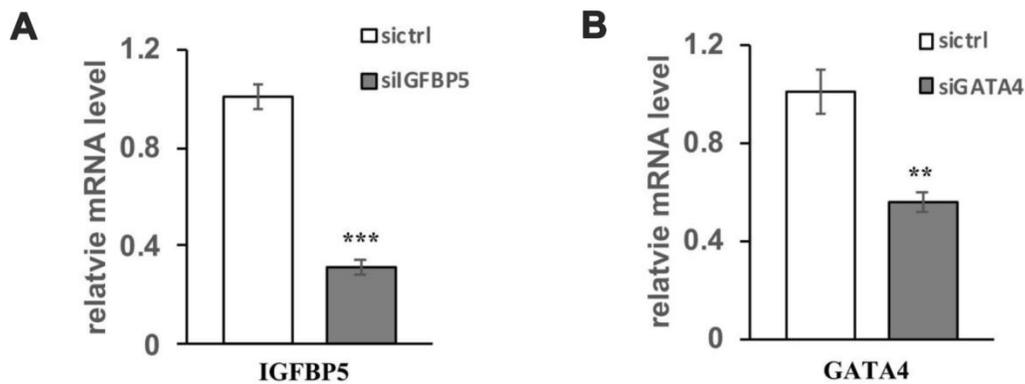
**Supplementary Figure 4. Y-27632 enhances expression of senescence markers p16 and p21 in commercial HDFs at 12 h after treatment.** (A, B). Commercial HDFs were treated with PBS or 10  $\mu$ M Y-27632, then were collected at the indicated times for analysis of p16 and p21 mRNA expression levels by RT-qPCR. All experiments were repeated at least 3 times, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$  when compared to the corresponding control as indicated.



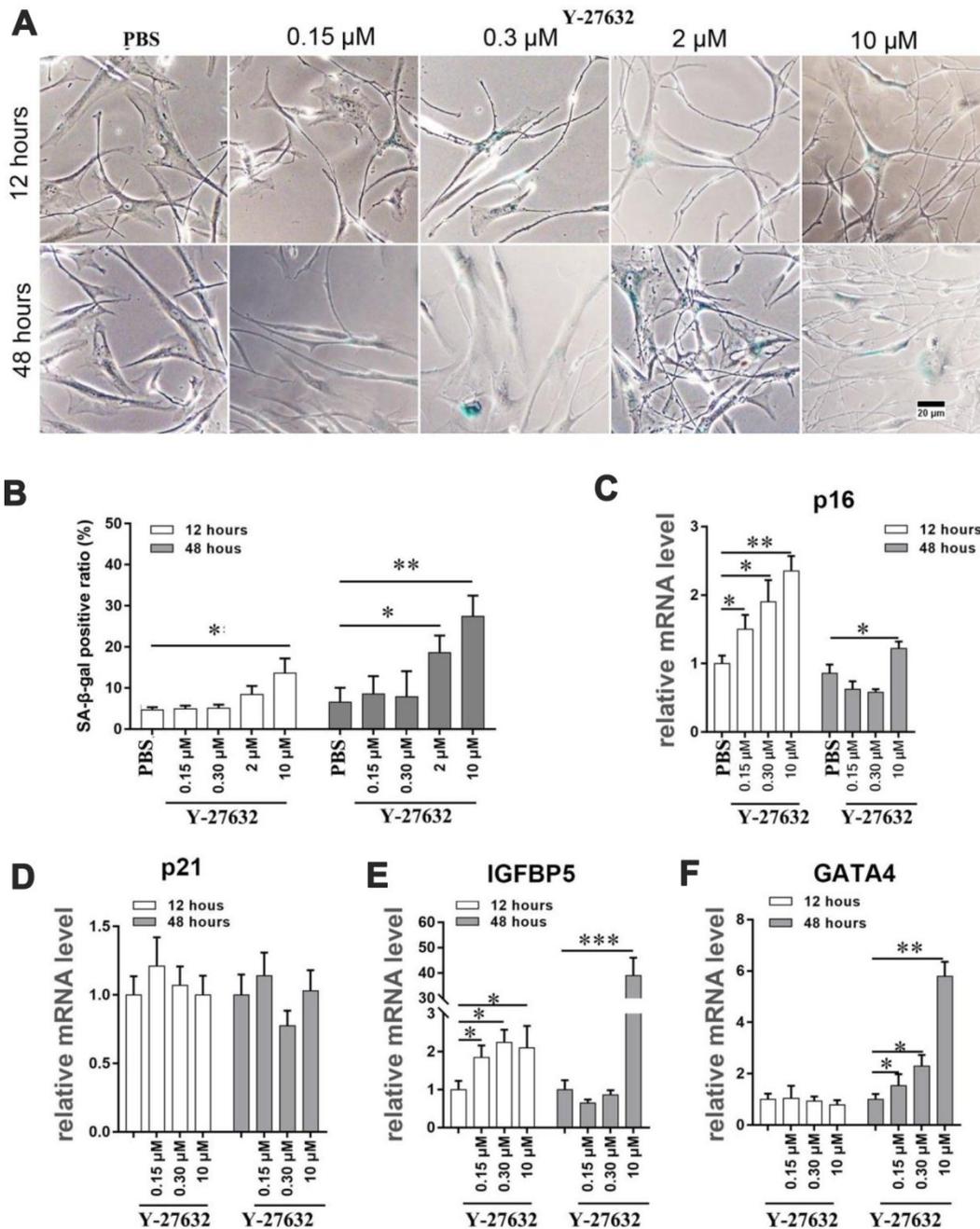
**Supplementary Figure 5. RNA-seq analysis of different gene expression profiles and related pathways of HDFs treated with or without Y-27632.** (A) The number of DEGs in HDFs treated with Y-27632 at 12 h (DY12) and 48 h (DY28) versus their corresponding controls (DNC12 and DNC48); 36 of those DEGs appeared at both time points. (B) All DEGs are shown in a heatmap and were divided into four clusters as indicated. DEGs in cluster 1 were downregulated both in Y+ and Y- at 12 h. DEGs in cluster 2 were downregulated only in both Y-27632 treated groups. DEGs in cluster 3 were upregulated only after Y-27632 treatment at 48 h. DEGs in cluster 4 were downregulated after Y-27632 treatment at 12 h and 48 h. (C) Principal component analysis (PCA) of the 4 groups based on the expression of all DEGs.



**Supplementary Figure 6. Prolonged treatment with Y-27632 induces the expression of IGFBP-5 and GATA4 in commercial HDFs.** (A, B) Commercial HDFs were treated with PBS or 10  $\mu$ M Y-27632, then were collected at the indicated times for analysis of IGFBP-5 and GATA4 mRNA expression levels by RT-qPCR. All experiments were repeated at least 3 times, \*\* $P < 0.01$ , \*\*\* $P < 0.005$  when compared to the corresponding control as indicated.



**Supplementary Figure 7. IGFBP-5 and GATA4 was knocked down efficiently by their corresponding siRNAs.** (A, B) HDFs were transfected with siRNAs, which were mixed with 3 independent siRNAs, targeting either IGFBP-5 or GATA4 and with a scrambled siRNA as a control (sictrl) and 48 h after transfection, mRNA levels of IGFBP-5 or GATA4 were analyzed using RT-qPCR. The fold change of mRNA levels in the siRNA-transfected HDFs relative to control HDFs (expression level as 1) is shown. All experiments were repeated at least 3 times, \*\* $P < 0.01$ , \*\*\* $P < 0.005$  when compared to the corresponding control as indicated.



**Supplementary Figure 8. Low concentrations of Y-27632 don't enhance cellular senescence in HDFs, but do induce the expression of p21, IGFBP-5 and GATA4.** (A, B) HDFs were treated with different concentrations of Y-27632 as indicated or PBS as a control, and were fixed at the indicated times and analyzed using a SA-β-gal staining kit to detect senescent cells. scale bar = 20 μm. (b) Quantification of (a), the percentage of SA-β-gal-positive cells (blue) was calculated based on counting a total of 500 cells. (C–F) HDFs were treated with different concentrations of Y-27632 as indicated or PBS as a control, and cells were collected at 12 and 48 h to analyze mRNA expression levels of p16, p21, IGFBP-5 and GATA4 by RT-qPCR. All experiments were repeated at least 3 times, \*P<0.05, \*\*P<0.01, \*\*\*P<0.005 when compared to the corresponding control as indicated.